a simple model for the reversal of the CPX clamp by Synaptotagmin and calcium. In this model, Synaptotagmin binds the stalled CPX-SNARE complex via its interactions with the t-SNAREs and upon binding Calcium, the membrane loops inserts rapidly into the bilayers, while Synaptotagmin stays bound in place on the SNAREpins. This is expected to perturb the attached SNAREpin out of the planarity of the zigzag array and thus, trigger the rapid disassembly of the stalled zigzag structure allowing the SNAREs to complete zippering and open a fusion pore.

2546-Pos Board B565

The Dorsal Root Ganglion Sandwich Synapse: Novel Transglial Signaling between Neuronal Somata

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The dorsal root ganglion (DRG) contains a subset of closely-apposed neuronal somata (*NS*) that are separated solely by a thin satellite glial cell (*SGC*) membrane septum to form a *NS-glial cell-NS* (*NGlN*) cell trimer. We recently reported that stimulation of one *NS* evokes a delayed, noisy and long-duration inward current in both itself and its passive partner that was blocked by suramin, a general purinergic antagonist. Here we test the hypothesis that *NGlN* transmission involves purinergic activation of the *SGC* and its release of an excitatory transmitter. Stimulation of the NS triggered a sustained current noise in the *SGC*. Block of transmission through the *NGlN* by reactive blue 2 or thapsigargin, aCa^{2+} store-depletion agent, implicated a Ca^{2+} store discharge-linked P2Y receptor. P2Y2 was identified by simulation of the *NGlN*-like transmission by puff of UTP onto the *SGC*. Block of the UTP effect by BAPTA, an intracellular Ca^{2+} scavenger, supported the involvement of *SGC* Ca^{2+} stores in the signaling pathway. The response to UTP was also blocked by AP5, which, along with the N2B subunit-specific antagonist ifenprodil, inhibited *NGlN* transmission, implicating a glutamatergic pathway via postsynaptic NMDA receptors. Puff of glutamate could evoke transmission-like current in the *NS*. Immunocytochemistry localized the NMDA receptor subunit NR2B to the *NS* membrane, abutting staining for P2Y2 on the *SGC* septum. We infer that *NGlN* transmission involves secretion of ATP from the *NS*, *SGC* Ca^{2+} store discharge via P2Y2 receptors and release of glutamate to activate *NS* postsynaptic NMDA receptors. Thus, the *NS* of the *NGlN* trimer communicate via a ''Sandwich Synapse'' transglial pathway, a novel signaling mechanism that may contribute to information transfer in other regions of the nervous system.

2547-Pos Board B566

The Interactions of Dopamine and L-Dopa with Lipid Headgroup and its Implication for Neurotransmitters Metabolism

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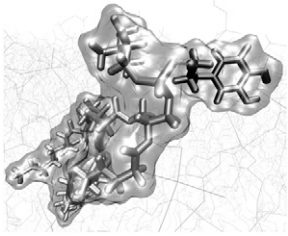
Atomistic molecular dynamics simulations were used to investigate the interactions between the neurotransmitter dopamine and its precursor L-dopa with membrane lipids. The results indicate that these molecules strongly interact with the lipid head groups e.g. via hydrogen bonds. These interactions anchor the dopamine and L-dopa to the membrane interfacial region. The strength of this bonding is dependent on lipids present in membrane. Presence of phosphatidylserine resulted in increased level of bonding strength with a lifetime longer than the timescale of our simulations.

The high membrane association of dopamine and L-dopa both, extracellularly, might favour the availability of these compounds for cell membrane uptake processes and, intracellularly, can accentuate the importance of membrane-bound metabolizing enzymes over their soluble counterparts.



We can also hypothesize that excessive association of dopamine and its precursor L-dopa with the membranes in the situation, when the concentration of phosphatidylserine is increased, may eventually limit the free use of dopamine as a synaptic transmitter what could possibly be a molecular level mechanism responsible for some of neurodegenerative disorders.

2548-Pos Board B567

Mapping the Energy Landscapes of the Glycine Receptor in the Post-Synaptic Neuronal Membrane

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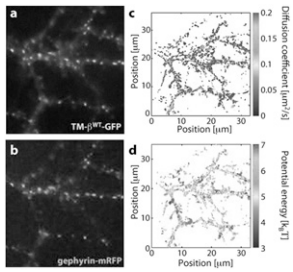
The movement of proteins in the cell membrane is governed by the local friction and their interactions with molecular partners. Yet, most experimental descriptions fail to unequivocally distinguish these effects; instead, they combine the diffusive and energetic contributions into an effective diffusion coefficient or anomalous exponent. Here, we show how the diffusion and energy landscapes of membrane proteins can be mapped separately over the entire cell surface using high-density single-molecule imaging and statistical inference [1]. In the case of glycine neuroreceptors, we demonstrate that scaffolds at inhibitory synapses act as energy traps with a depth modulated by the properties of the intracellular loop that mediates the receptor-scaffold interactions. Furthermore, we bridge the gap between local properties of the membrane environment and characteristics of the mobility at the cellular scale by simulating random walks in the inferred maps and computing estimators such as the propagator, mean square displacement, and first-passage time. Results are used to investigate the relation between numbers of receptors and synaptic plasticity. Overall, our approach provides a versatile framework with which to analyze biochemical interactions in situ.



[1] J.-B Masson et al, Nat. Chem. (submitted)

2549-Pos Board B568

High Precision Release of Neurotransmitter - A New Tool

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Modern imaging techniques have become a powerful tool for investigating excitation patterns and signaling pathways in the brain. However, the high complexity of brain topology and the resolution limit of in vivo techniques make it difficult to study isolated small neuron circuits. Micro-electrode arrays (MEAs) are able to record activity of in vitro neuron networks and to stimulate locally, but such electrical approach cannot easily be applied to stimulate single cells. Large stimulus artifacts and poor control over the spread of electrical stimuli in medium create the main disadvantages when studying the role of single cells in small networks. One promising solution is to interact with individual neural cells by mimicking chemical signaling. Recently developed systems for precise neurotransmitter release include micropipettes, microfluidic substrates and caged compounds.

In this project we propose to combine MEA technology (Multi Channel Systems GmbH, Switzerland) with novel FluidFM technology. The FluidFM (Cytosurge AG, Switzerland) has hollow atomic-force microscopy (AFM) cantilevers acting as force-controlled nanopipettes. We present the ability to locally release neurotransmitters on the cell membrane with precise control over applied force (sub-nN) and spatial position (μm). For those experiments we used FluidFM cantilevers with 2 μm openings, where the microfluidic channel was filled with physiological solution containing 200 μM glutamate. In order to chemically induce a local electrical response in a culture of dissociated hippocampal neurons from E18 rat, we first brought the FluidFM cantilever in contact with the cell membrane. We then applied pressure-pulses between 50 mbar and 300 mbar with 300 ms duration to locally eject sub picoliter volumes of the neurotransmitter.

We are now working towards applying single cell stimulations to well defined networks.

2550-Pos Board B569

Cocaine Preferentially Potentiates Fast Releasable Vesicle Pool in Mouse Dopaminergic Striatum *In Vivo*

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